# Timing of Rotavirus Vaccine Doses and Severe Rotavirus Gastroenteritis Among Vaccinated Infants in Low- and Middle-income Countries

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Applications to access the GSK and Merck and Co, Inc. clinical trial data can be submitted to ClinicalStudyDatarequest.com and http://engagezone.msd.com/ds_documentation.php, respectively. Computing code can be made available by the first author.

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**Background:** Altering rotavirus vaccine schedules may improve vaccine performance in low- and middle-income countries. We analyzed data from clinical trials of the monovalent (RV1) and pentavalent (RV5) rotavirus vaccines in low- and middle-income countries to understand the association between vaccine dose timing and severe rotavirus gastroenteritis incidence.

**Methods:** We assessed the association between variations in rotavirus vaccine administration schedules and severe rotavirus gastroenteritis risk. We used the complement of the Kaplan–Meier survival estimator to estimate risk differences for different schedules. To adjust risk differences (RDs) for confounding, we calibrated estimates in the vaccinated arm using estimates from the placebo arm.

**Results:** There were 3,114 and 7,341 children included from the RV1 and RV5 trials, respectively. The 18-month adjusted severe rotavirus gastroenteritis risk was 4.0% (95% confidence interval [CI] = 1.1, 7.1) higher for those receiving their first RV5 dose at <6 versus ≥6 weeks. For RV1, there was a 4.0% (95% CI = 0.0, 8.2) increase in 12-month adjusted risk for a 4- versus 6-week interval between doses. Further analysis revealed those receiving their first RV5 dose at 3–4 and 5–7 weeks had 2.9% (95% CI = 0.8, 5.3) and 1.3% (95% CI = −0.3, 3.0), respectively, higher risk compared with those at 9–12 weeks. Those receiving their first dose at 8 weeks had the lowest risk (RD: −2.6% [95% CI = −5.4, −0.1]) compared with those at 9–12 weeks.

**Conclusions:** A modest delay in rotavirus vaccination start and increase in interval between doses may be associated with lower severe rotavirus gastroenteritis risk in low- and middle-income countries.

**Keywords:** developing countries, gastroenteritis, infants, rotavirus, vaccines, vaccine schedules

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Despite the success of rotavirus vaccines at reducing healthcare encounters including hospitalizations,[1–3] rotavirus vaccine effectiveness remains lower in low- and middle-income countries than in high-income countries.[4,5] Many factors may contribute this lower vaccine effectiveness including concomitant vaccination with oral polio vaccine,[6,7] malnutrition,[8,9] interference by transplacental maternal antibodies,[10,11] and environmental enteropathy and the infant microbiota.[12–14]

Some factors shown to decrease rotavirus vaccine performance may be overcome by altering rotavirus vaccine schedules. A few studies have investigated the influence of vaccine schedules on vaccine performance,[10,11,15] but these studies have generally been restricted to immunologic endpoints. Because there is no known correlate of protection for antirotavirus immunoglobulin A (IgA) levels,[16,17] there is still uncertainty about the effect alternative schedules have on clinical endpoints. No data have been reported on the effect of timing of commonly used rotavirus vaccines as they are routinely administered (i.e., two doses of the monovalent [RV1, Rotarix; GlaxoSmithKline Biologicals, Rixensart, Belgium] and three doses of the pentavalent [RV5, RotaTeq; Merck & Co, Inc.; Kenilworth, NJ] rotavirus vaccines) using clinical endpoints.

We analyzed data from two large rotavirus vaccine trials to understand the association of timing of rotavirus vaccine doses and incidence of severe rotavirus gastroenteritis among children in low- and middle-income countries.

## METHODS

### Parent Study Data

This analysis used data from two randomized, phase III, placebo-controlled, multicenter clinical trials of RV1 and RV5 (Clinical Trial Number: NCT00241644 and NCT00362648, respectively) conducted in low- and middle-income countries. The trials have been described in depth elsewhere,[18–20] but a brief summary of each trial is below.

The RV1 clinical trial was conducted in South Africa and Malawi from 2005 to 2009. Healthy infants aged 5–10 weeks were enrolled and randomized to receive three placebo doses, a placebo dose followed by two RV1 doses, or three RV1 doses at approximately 6, 10, and 14 weeks of age. Enrolled infants were actively followed for occurrence of gastroenteritis from enrollment until study conclusion at 1 year of age with a subset followed for up to 2 years of age. Study staff visited parents or guardians at the homes of participants weekly to collect diary cards and also visited health clinics serving the study population. Stool samples were collected and tested for rotavirus using an enzyme-linked immunosorbent assay (Rotaclone, Meridian Biosciences, Cincinnati, OH) followed by reverse transcriptase polymerase chain reaction (RT-PCR) confirmation. Gastroenteritis was defined as three or more, looser than normal stools within a 24-hour period. Rotavirus gastroenteritis severity was determined using the 20-point Vesikari score.[21]

The RV5 study was conducted in Ghana, Kenya, Mali, Bangladesh, and Vietnam from 2007 to 2009. Infants 4–12 weeks of age were enrolled and randomly assigned to receive three doses of vaccine or placebo at approximately 6, 10, and 14 weeks of age. During the study, there was active surveillance for gastroenteritis at local clinics and hospitals. Any participant presenting with gastroenteritis provided a stool sample for testing of rotavirus using an enzyme immunoassay followed by RT-PCR confirmation. Gastroenteritis was defined as three or more watery or looser than normal stools within a 24-hour period or forceful vomiting. rotavirus gastroenteritis severity was classified using the 20-point modified Vesikari score.[21–23]

### Study Data

We analyzed data from the placebo and vaccinated arms of each trial as they are recommended for use (two doses of RV1 and three doses of RV5). In the RV1 trial, we included infants randomized to receive placebo or two doses of RV1 (precise schedules were three placebo doses or one placebo dose and two RV1 doses). In the RV5 trial, we included infants randomized to receive three doses of placebo or three doses of RV5. Each trial was analyzed separately, but the results are presented in parallel. This research was approved by the University of North Carolina at Chapel Hill Institutional Review Board (15–0401).

### Study Design Overview

We used a modified version of the study design described by Hernán et al[24] to understand whether timing of rotavirus vaccine doses, which was not randomized, was associated with risk of severe rotavirus gastroenteritis. We began by defining five different aspects of vaccine timing. For a specific aspect of timing (e.g., timing of first dose), we compared two or more predefined schedules related to that aspect of timing (e.g., first dose given at <10 vs. ≥10 weeks). We compared the schedules by assessing the cumulative risk of severe rotavirus gastroenteritis on an age-specific time scale to include severe episodes from 12 weeks of age. We did this by partitioning the follow-up time of infants such that infants could contribute person-time and severe events to more than one of the predefined schedules so schedules with delayed vaccine doses included severe episodes after 12 weeks of age in their estimated associations. After partitioning follow-up time and events, we estimated the association between each schedule and incidence of severe rotavirus gastroenteritis. We used the estimated associations of schedules in the placebo arm, which should have a null effect, to calibrate the estimates within the rotavirus-vaccinated arm to obtain adjusted estimates of the associations. This approach is similar to the negative control design described by Tchetgen Tchetgen.[25]

### Defining Schedules

We created and compared simplified schedules to understand the aspects of timing that may be associated with risk of severe rotavirus gastroenteritis. Because of the numerous combinations of schedules received, we could not simply

compare the schedules received in each trial. Instead, we classified the timing of doses a priori using five main aspects of dose timing: (1) timing of first-dose holding interval(s) between doses constant at 4 to 6 weeks, (2) timing of first dose, (3) timing of last dose, (4) length of interval(s) between doses, and (5) number of doses received at ≥10 weeks of age. For each aspect of timing, we defined and compared two or more schedules. All schedules were developed based on biologic plausibility, the potential for realistic interventions (e.g., alterations in rotavirus schedules that would fit at times routine vaccines are given as part of the Expanded Program on Immunization), and the nature of the data. The schedules for each aspect of timing are specified in Table 1 and a detailed description of each schedule can be found in eTable 1 (http://links.lww.com/EDE/B397). Owing to the number of associations estimated, we chose our primary aspect of timing a priori to be the timing of the first dose holding interval(s) between doses constant at 4–6 weeks. Completed weeks of age were used for all schedule definitions (e.g., 6 weeks and 5 days of age was categorized as 6 weeks of age).

## Outcome

We classified the outcome, first episode of severe rotavirus gastroenteritis, as infants experiencing gastroenteritis with a Vesikari or modified Vesikari score of ≥11. For all analyses, we analyzed data on an age-specific time scale with

**TABLE 1.** Predefined Rotavirus Vaccine Schedules for Each Aspect of Dose Timing

| Aspect of Timing | N[a] | N[b] | RV Type | Age at Dose 1 (Weeks) | Timing of Dose 2 | Timing of Dose 3 |
|---|---|---|---|---|---|---|
| First dose with 4–6 weeks between doses | 1,167 | 1,073 | RV5 | 3–6 | 4–6 weeks after first dose | 4–6 weeks after second dose |
| | 1,669 | 1,516 | RV5 | 7–9 | 4–6 weeks after first dose | 4–6 weeks after second dose |
| | 784 | 720 | RV5 | 10–12 | 4–6 weeks after first dose | 4–6 weeks after second dose |
| | 1,299 | 1,212 | RV1 | 10–12 | 4–6 weeks after first dose | NA |
| | 207 | 166 | RV1 | 13–16 | 4–6 weeks after first dose | NA |
| First dose | 519 | 506 | RV5 | <6 | ≤10 weeks after first dose | ≤10 weeks after second dose |
| | 3,147 | 3,063 | RV5 | ≥6 | ≤10 weeks after first dose | ≤10 weeks after second dose |
| | 2,882 | 2,805 | RV5 | <10 | ≤10 weeks after first dose | ≤10 weeks after second dose |
| | 784 | 764 | RV5 | ≥10 | ≤10 weeks after first dose | ≤10 weeks after second dose |
| Last dose | 1,527 | 1,216 | RV5 | ≤7 | ≤11 weeks of age | ≤15 weeks of age |
| | 3,520 | 2,353 | RV5 | ≤12 | ≤10 weeks after first dose | >15 weeks of age and ≤10 weeks after second dose |
| | 1,048 | 420 | RV1 | ≤11 | ≤15 weeks of age | NA |
| | 1,559 | 1,056 | RV1 | ≤16 | >15 weeks of age & ≤ 10 weeks after second dose | NA |
| Interval between doses | 2,589 | 1,310 | RV5 | ≤12 | 4 weeks after first dose | 4 weeks after second dose |
| | 3,345 | 1,453 | RV5 | ≤12 | 4 or 5 weeks after first dose[c] | 4 or 5 weeks after second dose[c] |
| | 3,474 | 546 | RV5 | ≤12 | 4, 5, or 6 weeks after first dose[d] | 4, 5, or 6 weeks after second dose[d] |
| | 1,559 | 337 | RV1 | ≤16 | 4 weeks after first dose | NA |
| | 1,559 | 926 | RV1 | ≤16 | 5 weeks after first dose | NA |
| | 1,559 | 167 | RV1 | ≤16 | 6 weeks after first dose | NA |
| Number of doses ≥ 10 weeks of age[e] | 1,030 | 2 | RV5 | <10 | <10 weeks of age | <10 weeks of age |
| | 3,666 | 397 | RV5 | <10 | <10 weeks of age | ≥10 weeks of age and ≤32 weeks of age |
| | 3,271 | 2,432 | RV5 | <10 | ≥10 weeks of age and ≤32 weeks of age | ≥10 weeks of age and ≤32 weeks of age |
| | 784 | 765 | RV5 | ≥ 10 | ≥10 weeks of age and ≤32 weeks of age | ≥10 weeks of age and ≤32 weeks of age |

[a]Number of infants beginning follow-up at 12 weeks of age in this schedule. Total sample size across schedules for an aspect of timing can sum to more than the total number of vaccinated infants in each trial because infants can begin in >1 schedule.
[b]Number of infants being followed for severe rotavirus gastroenteritis for a particular schedule at 6 months of age.
[c]At least one interval between the three doses must be 5 weeks.
[d]At least one interval between the three doses must be 6 weeks.
[e]Other timing of doses resulting in the same number of doses received ≥10 are possible and are included in eTable 1.
NA indicates not applicable.

follow-up beginning at 12 weeks of age (the latest time any infant enrolled in either trial). As mentioned above, we partitioned the follow-up time of infants such that infants could contribute person-time and severe gastroenteritis events to more than one schedule until the timing of their actual doses deviated from the schedule(s). This approach allowed us to include any severe events occurring after 12 weeks of age. Figure 1 presents three hypothetical infants and how their person-time and severe rotavirus gastroenteritis episodes were partitioned for timing of last dose. In this example, all infants contributed person-time to both predefined schedule until the actual timing of their last doses deviated from the predefined schedule. For example, infant A experienced a severe episode at 14 weeks of age, and this event was included in both schedules, because at 14 weeks of age, the timing of infant A's last dose was consistent with both predefined schedules. Additional details of the approach are included in eTable 2 (http://links.lww.com/EDE/B397).

## Covariates

We categorized covariate data on demographic information; breastfeeding and growth status; and infection status, antibiotic use, and other vaccinations. We categorized growth status using the World Health Organization criteria.[26] We classified infection status, antibiotic use, and concomitant vaccination using medical histories.

## Statistical Analysis

To estimate the association between timing of rotavirus vaccine doses and incidence of severe rotavirus gastroenteritis, we estimated risk differences (RDs) and risk ratios (RRs) between different schedules for each aspect of timing at 6, 12, and 18 months of age using cumulative risk estimates obtained from the complement of the Kaplan–Meier survival estimator at those time points.[27] We chose a priori to focus on 12 months of age as our primary time point of interest, because that provided adequate time for severe gastroenteritis events to occur while allowing a majority of participants to remain in the cohorts. We did not estimate RDs or RRs at specific time points if any schedule had fewer than five severe events at that time point. We also estimated hazard ratios using Cox proportional hazard models.

There was potential for bias in the association between dose timing and severe rotavirus gastroenteritis, because of confounding and administrative censoring in the study design. Data from the placebo arms was used as a negative control to adjust for both potential sources of bias. Because the timing of placebo doses should not influence the incidence of severe rotavirus gastroenteritis in the placebo arm, any association observed would be owing to bias. This association provided an estimate of amount of bias expected from uncontrolled confounding within the vaccinated arms, assuming potential uncontrolled confounders influencing the timing of receipt of doses in the placebo arms were the same confounders as those in the rotavirus-vaccinated arms. Associations in the placebo arm were used to calibrate (i.e., adjust) the estimates among those in the vaccinated arm. The directed acyclic graphs in eFigure 1 (http://links.lww.com/EDE/B397) provide a conceptual diagram of this approach.

Before calibrating estimates, we empirically verified that imbalances in measured covariates between schedules were similar in the placebo and vaccinated arms using standardized mean differences. Standardized mean differences were calculated as $(p1 - p2)/[\{p1(1 - p1) + p2(1 - p2)\}/2]^{1/2}$, where $p1$ was the proportion (or mean) of the binary covariate for a specific schedule (e.g., first dose at <6 weeks), and $p2$ was the proportion in a different schedule (e.g., first dose at ≥6 weeks). If the imbalance in covariates was similar (<10% difference) between the placebo and vaccinated arms, we assumed calibration of the estimates in the vaccinated arm would yield a sufficiently adjusted estimate of the associations.

To calibrate (i.e., adjust) the associations of rotavirus vaccine dose timing, we estimated RD and RR measures comparing schedules for each aspect of timing, as described above, for both the placebo and vaccinated arms of each trial.

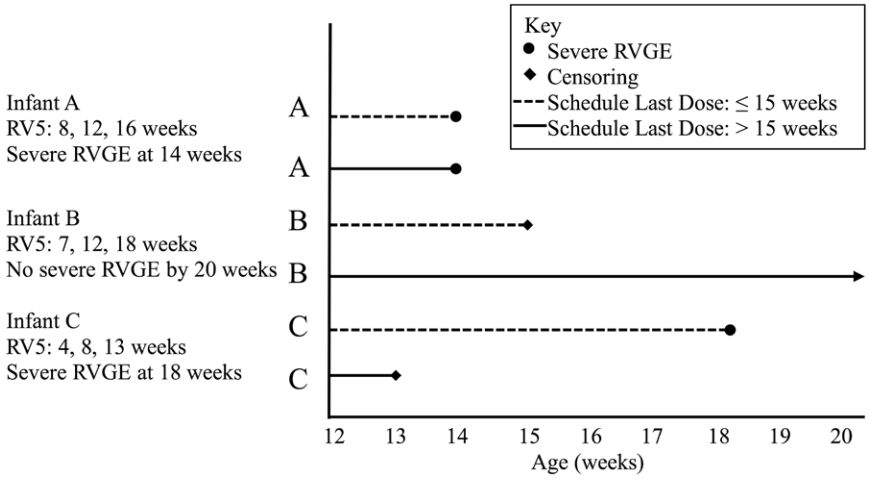



**FIGURE 1.** Example of the person-time and event categorization for three hypothetical infants. RVGE indicates rotavirus gastroenteritis.

We then calibrated the estimates among those vaccinated with the estimates among those in the placebo arms by subtracting the difference measures and dividing the ratio measures (i.e., difference in differences and ratio of ratios).[28] A nonparametric bootstrap with 2,000 sample draws with replacement was used to obtain the point estimates and 95% empirical confidence intervals.[29] The median of the distribution of calibrated estimates was reported for the difference and ratio estimates, and the 2.5th and 97.5th percentiles of the distribution were reported for the lower and upper bounds of the 95% empirical confidence intervals.

We conducted additional post hoc analyses to assess the association between timing of first dose and incidence of severe rotavirus gastroenteritis. We estimated the 12-month RD of severe gastroenteritis by week of age at first dose, requiring ≥100 infants in each stratum. We then collapsed across ages with similar RDs to estimate the RDs more precisely. We also estimated the 12-month RD between a 6/10/14 and 8/12/16 week schedule for RV5. All analyses were performed using SAS Clinical Trial Data Transparency (version 4.5.2; SAS Institute, Inc., Cary, NC).

## RESULTS

There were 3,114 and 7,341 children included in this analysis from the RV1 and RV5 trials, respectively (Table 2). Infants were followed for a median time of 286 and 301 days after 12 weeks of age in both the placebo and RV1-vaccinated arms, respectively. Median follow-up was 483 days after 12 weeks of age in both the placebo and RV5-vaccinated arms. A total of 154 (100 and 54 in placebo and RV1 arms, respectively) and 324 (205 and 119 in placebo and RV5 arms, respectively) severe rotavirus gastroenteritis events occurred in the RV1 and RV5 trials, respectively.

The distributions of measured covariates between schedules for each aspect of timing were similar for the placebo and rotavirus-vaccinated arms (data available upon request). This provided empirical evidence for our assumptions for using calibration for adjustment. However, one comparison, first dose of RV1 with 4–6 weeks between doses, had a number of deviations, which may indicate presence of residual confounding.

### First Dose With 4–6 Weeks Between Doses

The adjusted risk of severe rotavirus gastroenteritis was about 2% higher for those infants receiving their first RV5 dose at 3–6 weeks of age compared with 10–12 weeks of age (holding intervals between all subsequent doses at 4–6 weeks) [12-month RD: 2.1% (95% confidence interval [CI] = 0.1, 4.0); 12-month RR: 2.2 (95% CI = 0.7, 6.7)] (Figures 2 and 3).

### First Dose

The 18-month adjusted risk of severe rotavirus gastroenteritis was 4.0% (95% CI = 1.1, 7.1) higher for those receiving their first RV5 dose at <6 versus ≥6 weeks of age. The 18-month RR was 2.4 (95% CI = 1.3, 4.8).

### Last Dose

Infants receiving their last RV5 doses at ≤15 versus >15 weeks of age had almost a 2% increase in 12-month adjusted risk of severe rotavirus gastroenteritis (12-month RD: 1.9% [95% CI = 0.6, 3.2]; 12-month RR: 1.7 [95% CI = 0.7, 4.2]). This was slightly attenuated and less precise for RV1 (12-month RD: 1.4% [95% CI = −1.1, 4.1]; 12-month RR: 0.7 [95% CI = 0.1, 2.2]).

### Interval Between Doses

For RV1, there was a 4.0% (95% CI = 0.0, 8.2) increase in 12-month adjusted risk for a 4- versus 6-week interval between doses (12-month RR: 3.6 [95% CI = 0.9, 31.6]). This association was diminished to about 2% when comparing a 5- to 6-week interval, but this estimate was imprecise (12-month RD: 1.9% [95% CI = −2.2, 5.9]; 12-month RR: 4.5 [95% CI = 0.9, 46.5]).

### Number of Doses ≥10 Weeks of Age

Those receiving only one dose of RV5 at ≥10 weeks of age had a higher adjusted risk of severe rotavirus gastroenteritis compared with those receiving three doses at ≥10 weeks (12-month RD: 2.6% [95% CI = 0.7, 4.7]; 12-month RR: 4.8 [95% CI = 0.7, 75.3]). When the analysis comparing one versus three doses of RV5 at ≥10 weeks of age excluded infants missing the second or third dose of vaccine, the effect seen was similar (12-month RD: 2.5% [95% CI = 0.5, 4.7]; 12-month RR: 4.7 [95% CI = 0.7, 70.5]).

### Additional Results

The estimated uncalibrated cumulative risk of severe rotavirus gastroenteritis stratified by schedule and treatment status (i.e., placebo or vaccinated) is shown for all comparisons in eFigures 2–10 (http://links.lww.com/EDE/B397). Hazard ratios are presented in eFigure 11 (http://links.lww.com/EDE/B397) and were similar to RRs. Calibrated, uncalibrated, and placebo RDs and RRs at 12 and 18 months are presented in eFigures 12 and 13 (http://links.lww.com/EDE/B397), respectively. RDs and RRs were not estimated for any comparisons at 6 months of age and for first dose at <6 versus ≥6 weeks of age schedule at 12 months of age, because there were fewer than five events for at least one schedule.

The post hoc assessment of the dose–response relationship between timing of first dose and incidence difference of severe rotavirus gastroenteritis is presented in Figure 4. Those receiving their first RV5 dose at 3–4 and 5–7 weeks had 2.9% (95% CI = 0.8, 5.3) and 1.3% (95% CI = −0.3, 3.0), respectively, higher severe gastroenteritis risk compared with those at 9–12 weeks. Those receiving their first dose at 8 weeks had the lowest risk (RD: −2.6% [95% CI = −5.4, −0.1]) compared with those at 9–12 weeks. In addition, the estimated 12-month RD for a 6/10/14 compared with 8/12/14 week schedule of RV5 was 7.1% (95% CI = 2.9, 11.8).

## DISCUSSION

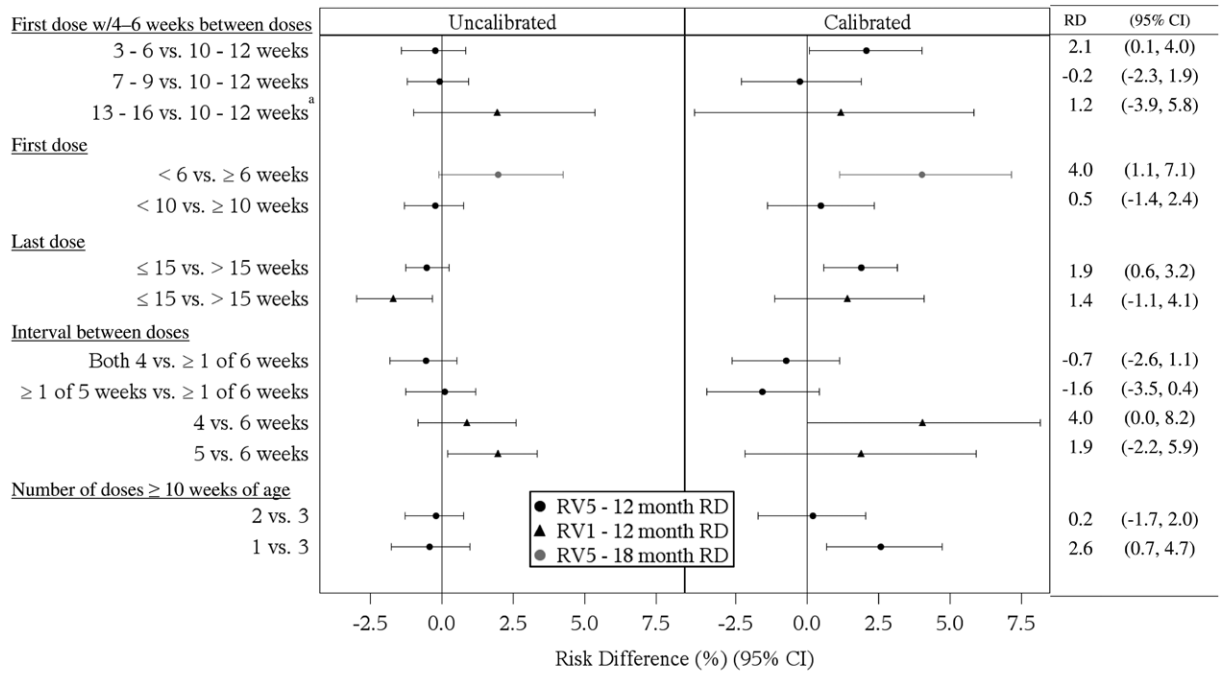
Comparisons from the RV5 trial indicated earlier vaccination of the first RV5 dose resulted in a higher risk of severe

**TABLE 2.** Characteristics of the Trial Populations

| Infant Characteristics | Trial 1 (N = 3,114) | | Trial 2 (N = 7,341) | |
|---|---|---|---|---|
| | RV1 (n = 1,560) | Placebo (n = 1,554) | RV5 (n = 3,666) | Placebo (n = 3,675) |
| Median length of follow-up in days from 12 weeks of age | 301 | 286 | 483 | 483 |
| First severe rotavirus gastroenteritis episode | 54 | 100 | 119 | 205 |
| Age in weeks at vaccine or placebo receipt | | | | |
| Dose 1[a], mean (SE) | 11.2 (0.03) | 11.3 (0.03) | 7.6 (0.03) | 7.5 (0.03) |
| Dose 2[b], mean (SE) | 16.2 (0.04) | 16.3 (0.04) | 12.2 (0.04) | 12.1 (0.04) |
| Dose 3[c], mean (SE) | — | — | 16.7 (0.04) | 16.7 (0.04) |
| Demographic | | | | |
| Female sex (%) | 48.8 | 48.6 | 48.6 | 49.7 |
| African race (%) | 97.2 | 96.8 | 72.3 | 72.4 |
| Asian race (%) | — | — | 27.6 | 27.6 |
| Growth status at enrollment | | | | |
| Stunted (%) | 22.6 | 21.7[d] | 10.1 | 10.4[e] |
| Underweight (%) | 3.9 | 4.4 | 11.5 | 11.2 |
| Wasting (%) | 3.7[d] | 4.3[d] | 23.1[d] | 20.9[d,e] |
| Exclusively breastfed | | | | |
| At dose 1 (%) | — | — | 80.0 | 81.6 |
| At dose 2 (%) | — | — | 75.2[d] | 74.7 |
| At dose 3 (%) | — | — | 69.9 | 70.1[d] |
| ≥1 infection(s) | | | | |
| At dose 1 (%) | 0.5 | 0.9 | 5.5 | 5.6 |
| At dose 2 (%) | 0.3 | 0.4 | 0.5 | 0.6 |
| At dose 3 (%) | — | — | 1.1 | 1.3 |
| ≥1 antibiotic used[f] | | | | |
| At dose 1 (%) | 12.9 | 14.1 | 2.2 | 2.8 |
| At dose 2 (%) | 15.5 | 14.6 | 5.6 | 5.2 |
| At dose 3 (%) | — | — | 6.2 | 6.1 |
| Routine vaccines | | | | |
| ≥1 BCG vaccine | | | | |
| At dose 1 (%) | — | — | 21.9 | 22.0 |
| ≥1 DTP-HB/HIB vaccine[g] | | | | |
| At dose 1 (%) | 99.3 | 99.3 | 45.7 | 47.0 |
| At dose 2 (%) | 99.1 | 99.5 | 43.6 | 43.8 |
| At dose 3 (%) | — | — | 42.0 | 42.2 |
| ≥1 oral polio vaccine | | | | |
| At dose 1 (%) | 99.3 | 99.3 | 54.1 | 55.6 |
| At dose 2 (%) | 99.2 | 99.5 | 51.1 | 51.0 |
| At dose 3 (%) | — | — | 48.2 | 48.1 |

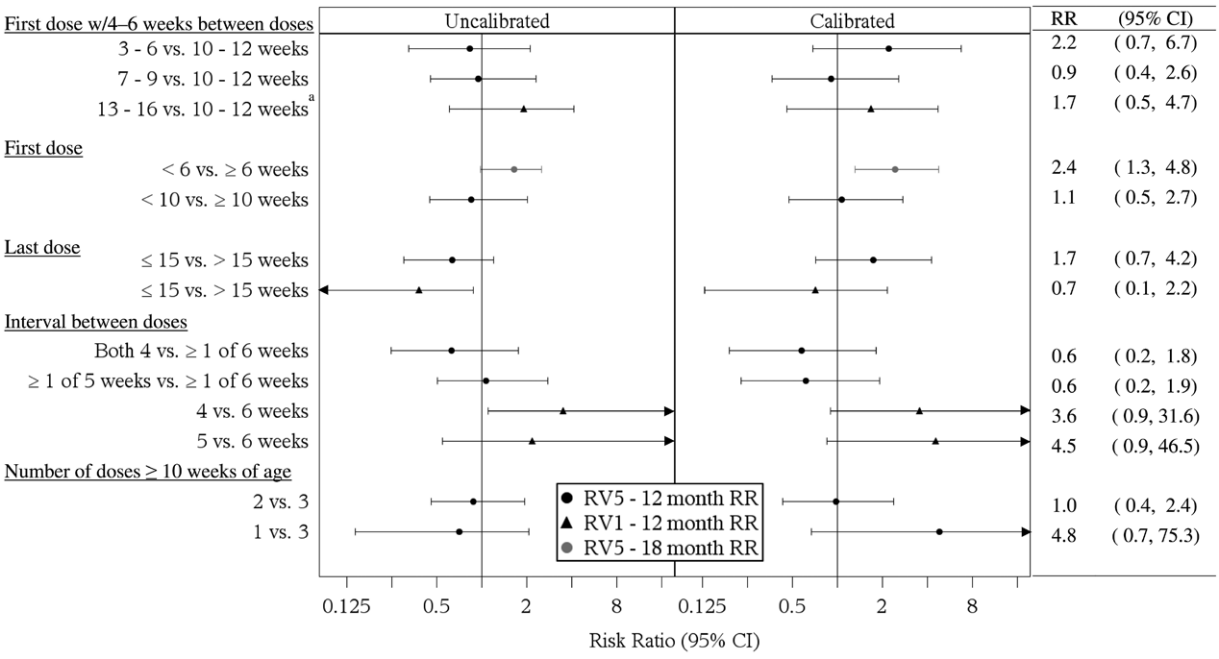
[a]There were 25 and 21 in the placebo and RV1 arms, respectively, missing dose 1.
[b]There were 63 and 45 in the placebo and RV1 arms, respectively, missing dose 2. There were 17 and 10 in the placebo and RV5 arms, respectively, missing dose 2.
[c]There were 57 and 47 in the placebo and RV5 arms, respectively, missing dose 3.
[d]Missing 1–15 observations, excluding those missing doses of vaccine or placebo.
[e] Excluding Bangladesh.
[f]Excluding topical antibiotics.
[g]Or DTaP & HB, which were the standard vaccines given in Asian countries.

BCG indicates Bacillus Calmette–Guérin; DTaP, diphtheria and tetanus toxoids and acellular pertussis; DTP-HB/HIB, diphtheria-tetanus-pertussis-Hepatitis B and -Haemophilus influenza B.




[a] Due to differences in the covariate patterns between the placebo and vaccine arms, the calibrated estimate should be interpreted with caution.

**FIGURE 2.** Uncalibrated and calibrated RDs and 95% CIs.




[a] Due to differences in the covariate patterns between the placebo and vaccine arms, the calibrated estimate should be interpreted with caution.

**FIGURE 3.** Uncalibrated and calibrated RRs and 95% CIs.

rotavirus gastroenteritis with this risk declining with later vaccination until approximately 8 weeks of age. Importantly, these associations were present even when accounting for the occurrence of severe gastroenteritis episodes after 12 weeks of age. In a previous analysis conducted by the first author, there was heterogeneity of vaccine efficacy (comparing vaccinated infants to unvaccinated infants) for those with a first dose of RV5 received at <8 weeks compared with ≥8 weeks

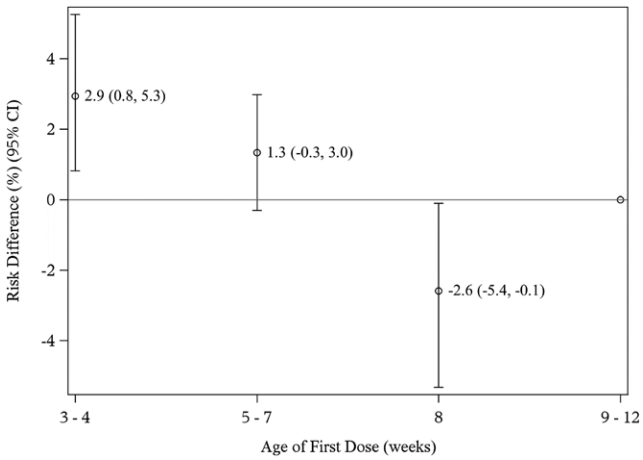



**FIGURE 4.** Post hoc analysis of 12-month calibrated RDs and 95% CIs for timing of first RV5 dose (referent 9–12 weeks).

of age.[9] This analysis found a similar pattern when accounting for severe gastroenteritis episodes after 12 weeks of age and comparing different schedules among vaccinated infants. The results were consistent with trials investigating alternative rotavirus schedules reporting immunologic endpoints.[30] Vaccination with RV1 at 10/14 versus 6/10 weeks of age resulted in higher seroconversion percentages. At 18 weeks of age, the seroconversion percent difference comparing a 10/14 versus 6/10 week schedule was 12.8% (95% CI = 1.2, 23.2) in Ghana and 10.1% (95% CI = −0.4, 20.3) in Pakistan.[10,11]

Lower incidence of severe rotavirus gastroenteritis with delays in first rotavirus vaccine dose could be owing to a number of biologic factors including a decline in transplacentally acquired antirotavirus immunoglobulin G (IgG) antibodies or changes in the microbiota that allow for a stronger immune response with slightly older ages at first dose. In Ghana and Pakistan, there was higher seroconversion among infants in the lowest compared with highest quartile of prevaccination IgG levels.[10,11] In addition, a case–control study of 6-week-old Ghanaian infants found RV1 vaccine responders (postvaccination IgA antibody levels ≥20 IU/ml) had microbiotas more closely resembling Dutch infants than Ghanaian nonresponders (postvaccination IgA antibody levels <20 IU/ml).[13] It may be possible such differences in microbiota between responders and nonresponders can be overcome with age, leading to improved vaccine response when vaccinated at slightly later ages. However, a study in Vellore, India, reported similar immunologic response to RV1 regardless of the presence of enteropathogens at the time of vaccination.[31] Therefore, the intestinal microbiota may not be the mechanism by which the association between timing of rotavirus vaccine dose and severe gastroenteritis is mediated.

We also found an increase in risk of severe rotavirus gastroenteritis for those infants with a 4-week interval between RV1 doses compared with a 6-week interval between doses. For RV5, there was no association between length of interval between doses and severe gastroenteritis. To our knowledge, no studies have investigated the association between interval length and outcomes for rotavirus vaccines. A study of intervals between oral polio vaccine doses in Bangladesh reported similar seroconversion proportions among those vaccinated with a 2- versus 4-week interval between doses.[32] However, seroconversion proportions for some poliovirus types were lower for the 2- versus 4-week intervals. A longer delay between doses may provide a greater booster effect for oral vaccines, including RV1. However, the underlying mechanism for the observed association was not investigated and remains unknown.

There were a number of limitations of this research. Because this was a reanalysis of previously collected data, there were restrictions on the comparisons we could assess. Also, we were unable to account for events that occurred before 12 weeks of age. In the RV5 cohort, no events occurred before 12 weeks of age, but three events did occur before 12 weeks of age in the RV1 trial (one and two in the placebo and RV1 arms, respectively). eTable 3 (http://links.lww.com/EDE/B397) provides estimates from the placebo arms of each trial of the expected number of severe rotavirus gastroenteritis episodes that would occur by delaying vaccination start by 2 or 4 weeks. We were also unable to stratify by continent or country owing to the relatively few number of severe gastroenteritis episodes. There may be important differences between low- and middle-income countries that we could not assess. In addition, there may have been residual confounding of some estimates that we were unable to account for in this analysis. Although the vast majority of schedules had similar covariate imbalances in the placebo and vaccinated arms, there were a few comparisons that did not deviate in a similar manner. This means that after calibration of estimates, these estimates may have had some residual confounding. In addition, because we were unsure of which aspect of timing could potentially be associated with risk of severe rotavirus gastroenteritis, we defined a priori a number of schedules to compare for each aspect of timing. These multiple comparisons may have resulted in observing associations by random chance.

Despite these limitations, there were a number of strengths of this study. The data from these trials allowed us to assess how the timing of rotavirus doses relates to the occurrence of severe rotavirus gastroenteritis, which has not been previously reported. The study had four notable advantages: (1) our study used data from two trials of two rotavirus vaccines to understand the association between rotavirus vaccine timing, (2) the sample sizes of the trials were large enough to assess severe gastroenteritis as the outcome of interest, (3) we were able to use a novel study design to include rotavirus episodes after 12 weeks of age to ensure later schedules were penalized for any early episodes that occurred before receipt of the vaccine, and (4) data in the placebo arms were leveraged as a negative control to adjust estimates of the association between timing of rotavirus vaccine doses and incidence of severe gastroenteritis.

In this reanalysis of two clinical trials of rotavirus vaccines in low- and middle-income countries, we found there was an association between rotavirus vaccine dose timing and incidence of severe rotavirus gastroenteritis. These data suggest a modest delay in RV5 start and increase in interval between RV1 doses, when the series begins at approximately 10 weeks of age, may improve rotavirus vaccines performance in low- and middle-income countries. However, the decision to delay rotavirus vaccination needs to be carefully considered in conjunction with early rotavirus exposures and potential missed vaccination opportunities to fully evaluate the potential benefits of delaying rotavirus vaccination start in low- and middle-income countries.

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